Fragmentation of a Novel Marine Peptide, Plicatamide, Involves an Unusual Gas-Phase Intramolecular Rearrangement

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During our characterization of plicatamide **1**, a modified octapeptide: Phe-Phe-His-Leu-His-Phe-His-dcΔDOPA (where dcΔDOPA = decarboxy-(*E*)-α,β-dehydro-3,4-dihydroxyphenylalanine) from the blood cells of the ascidian *Styela plicata*, we noted a series of fragment ions from the $[M + H]^+$ ion which could not be assigned. There was no evidence in the ^1H NMR spectrum to support an alternative molecular structure and the series of fragment ions were not present in the tandem mass spectrometry analysis of the $[M + Na]^+$ ion. In addition, there was no evidence that the sample was a mixture of isobaric compounds. We propose that an unusual C-terminal to N-terminal rearrangement is responsible for the series of fragment ions from the $[M + H]^+$ ion. This rearrangement was not observed in peptide analogs of plicatamide which did not contain the dcΔDOPA at the C-terminus suggesting that this moiety is critical for the rearrangement. The proposed reaction is analogous to that recently reported by Vachet et al. involving a fragment ion formed from leucine enkephalin. (J Am Soc Mass Spectrom 2001, 12, 470–474) © 2001 American Society for Mass Spectrometry

When characterizing a peptide of unknown sequence, mass spectrometry often plays an invaluable role. After chemical sequence analysis has been carried out, it is generally considered a necessity to obtain intact peptide mass analysis to complement the chemical sequence information [3, 4]. Even more critical is the case where Edman degradation based analysis results in only a partial sequence, or when modified amino acids are incorporated into the peptide sequence for which the phenylthiohydantoin (PTH) amino acids are neither stable nor easily analyzed. In these cases, mass spectrometry often provides the additional information required to fully characterize the peptide. For example, unless special precautions are taken, when γ-carboxy glutamic acid [5, 6], 6-bromotryptophan [7], or glycosylated residues [8] are encountered, the chemical sequence analysis for the corresponding cycle is blank. In the past we have used the high accuracy mass analysis available with Fourier transform mass spectrometry to resolve the ambiguities presented by the occurrence of these modified residues [9]. Alternatively, when insufficient material is available for complete chemical sequence analysis then tandem mass spectrometry (MS/MS) analysis has also been used to complement the chemical sequence analysis [7, 10].

Ideally, when interpreting the MS/MS spectrum of an unknown, all of the fragment ions observed would be assigned to single bond scissions derived from the proposed precursor structure. Clearly this is an idealized case, and quite often relatively low intensity fragments are observed in the MS/MS spectrum due to multiple bond scissions. Although multiple bond scission fragments are not generally helpful in determining an unknown sequence, they can be reconciled with the precursor structure once their origin is recognized [11]. However, multiple bond scission fragments are not the only type of fragment ion that can interfere with attempts to assign fragment ions. Intramolecular rearrangement reactions of a peptide ion occurring in a mass spectrometer have also been observed [2, 12–14]. Fortunately, these rearrangement ions are typically of low intensity and have been more of a curiosity recognized by physical chemists concerned with understanding gas phase ion reaction mechanisms.

In the pursuit of ever more sensitive mass and MS/MS analysis instruments, electrospray ionization has been coupled with the quadrupole ion trap mass spectrometer. Although this configuration has proven to have a number of significant advantages including

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sensitivity, it has also been recognized that there are potential disadvantages with the quadrupole ion trap [2]. The high sensitivity of this instrument in part reflects the advantages of electrospray as a method of converting a solution to gas phase ions and the ability of the ion trap to capture 100's to 1000's of ions of specific mass to charge ratio, and subsequently detect the 10's to 100's of fragment ions formed from the excitation of the precursor ions. However, the ability to trap ions particular for extended periods of time also increases the probability of internal rearrangement of ions [2]. As a result, when interpreting MS/MS spectra analyzed with ion trap instruments, multiple bond scissions and rearrangement ions can limit attempts to determine the sequence of an unknown peptide. In particular, the possibility of rearrangements which result in fragment ions which are not consistent with the proposed precursor structure must be recognized. Here we document a gas phase rearrangement reaction encountered when characterizing an unknown with MS/MS.

Experimental

Mass spectra were measured by directly infusing (1–2 μL/min) the reverse-phase-high-performance liquid chromatography (RP-HPLC) purified plicatamide sample diluted in 0.1% acetic acid in methanol (1:1) into an Esquire (Bruker Daltonics, Billerica, MA) ion trap mass spectrometer through an orthogonal microspray needle based on a modified fused silica capillary (Nu-Objectives, MA). The instrument was operated with −4 kV capillary potential and −0.5 kV endplate offset. Other conditions were standard with the exception that the fragmentation time for controlling the residence time of ions in the trap for the MS^3 experiments (including Figure 2) was increased from 40 to 200 ms.

Plicatamide was purified and characterized as previously described [1]. The decarboxylated dehydro DOPA residue was found to reside on the C-terminus of the peptide (E configuration) based on both ^1H NMR and Edman sequence analysis [1]. The homogeneity of the plicatamide sample was determined by acetic acid urea polyacrylamide electrophoresis (AU-PAGE) and RP-HPLC [1]. [Tyr^8] plicatamide (H-Phe-Phe-His-Leu-His-Phe-His-Tyr-OH) was purchased from Research Genetics and purified by RP-HPLC. [$DOPA^8$] plicatamide (H-Phe-Phe-His-Leu-His-Phe-His-DOPA-OH) was generated using a modification of the mushroom tyrosinase catalyzed hydroxylation reaction described by Marumo and Waite [15], the details of which will be reported elsewhere (Taylor, unpublished results).

Results and Discussion

Figure 1 compares the MS/MS spectra of the $[M + H]^+$ and $[M + Na]^+$ ions of plicatamide. The MS/MS spectrum of the $[M + H]^+$ ion contains intense series of a and b type fragment ions as shown in Figure 1A and Table 1. In the MS/MS of the $[M + Na]^+$ ion intense series of a, b, and y type ions were observed (see Figure 1B and Table 1). Based on the MS/MS of the $[M + Na]^+$ ion together with the chemical sequence and NMR analysis, the structure of plicatamide was determined [1].

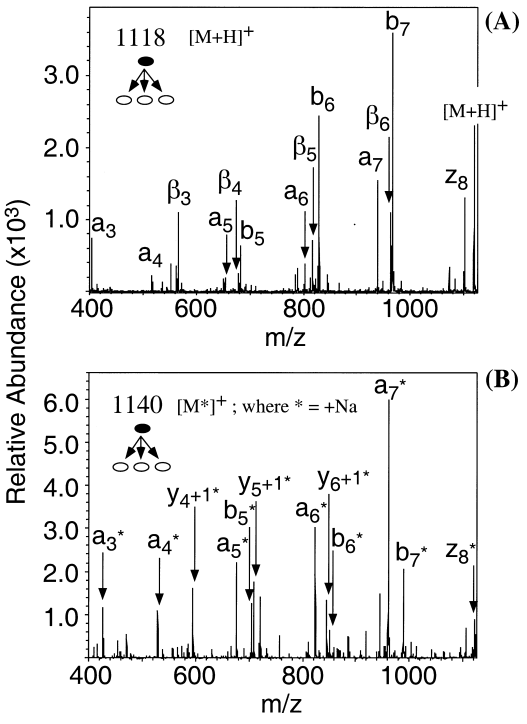


Figure 1. Compares the electrospray ion trap MS/MS spectra of the **(A)** $[M + H]^+$ and **(B)** $[M + Na]^+$ ions of plicatamide.

In the MS/MS of the $[M + H]^+$ ion the intense series of a and b type fragment ions were consistent with the proposed structure. However, in Figure 1A, an additional series of fragment ions are observed at *m/z* 566.3, 679.4, 816.7, and 963.6. This series of ions cannot easily be assigned based on the proposed structure. This series of ions, designated "β ions" was not present in the MS/MS of the $[M + Na]^+$ ion, nor were there unassigned fragment ions in the MS/MS of the $[M + Na]^+$ to suggest an impurity was present (see Table 1). The mass difference between successive β_3 to β_6 fragment ions was 113.1, 137.3, and 147.2 Da which corresponds with the mass of Leu, His, and Phe residues. In addition, the mass difference between the β_6 ion and the z_8 ion (*m/z* 1100.7) was 137.1 Da which corresponded with the mass of an His residue. Therefore the β_3 to β_6 and z_8 ions gave the C-terminal sequence Leu-His-Phe-His consistent with the C-terminus of plicatamide without the C-terminal decarboxy-(*E*)-α,β-dehydro-3,4-dihydroxyphenylalanine (dcΔDOPA) residue. Two possibilities were considered for the origin of this series of fragment ions: (i) that an unusual rearrangement occurs with the C-terminal dcΔDOPA residue recombining with the N-terminal phenylalanine to form species **2** with loss of ammonia (Scheme **1**) which could subsequently generate the β series of fragment ions or (ii) that

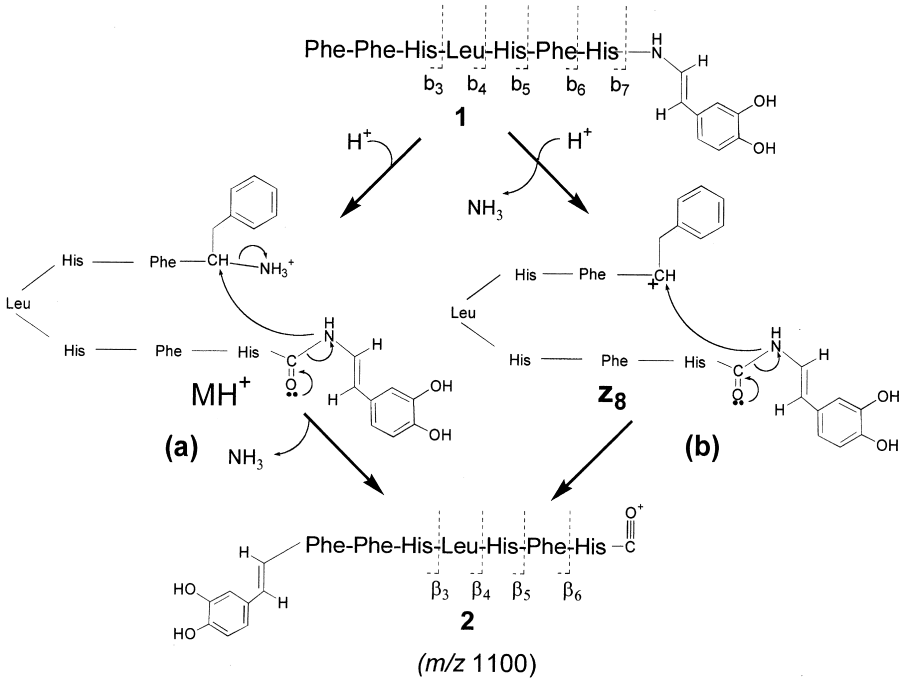
Table 1. Assignment of fragment ions observed from the MS/MS spectra of plicatamide and its analogs

Assignment	Plicatamide $[M + H]^+$	Plicatamide[b] $[M + Na]^+$	[DOPA[8]] plicatamide $[M + H]^+$	[Tyr[8]] plicatamide $[M + H]^+$
a_3	404.3	426.3	—	—
b_3	—[a]	454.3	—	—
$y_3 + 2$	—	458.2	—	—
$c_3 + 2$	—	471.3	—	—
$y_6 + 2/a_5$	—	529.4	—	—
a_4	517.4	539.4	—	—
β_3	566.3	—	—	—
b_4	—	567.3	—	—
$c_4 + 2$	563.3	584.4	—	—
$y_4 + 2$	573.3	595.4	619.0	603.2
$z_5 - 18$	651.6	—	—	—
a_5	654.6	676.5	655.6	654.3
β_4	679.4	—	—	—
b_5	682.5	704.4	682.6	682.4
$a_6 - 17$	784.5	—	—	—
$z_6 - 18$	788.6	—	—	—
$y_5 + 2$	—	708.5	732.5	716.2
$c_5 + 2$	—	721.4	—	—
a_6	801.4	823.7	801.6	801.0
$b_6 - 17$	812.7	—	—	—
β_5	816.7	—	—	—
b_6	829.7	851.7	829.5	829.5
$y_6 + 2$	—	845.6	869.4	853.4
a_7	938.5	960.7	938.4	938.5
β_6	963.6	—	—	—
b_7	966.7	988.7	966.5	966.5
$[M + H - 45]$	1072.5	1094.9	—	—
$[M + H - 35]$ or $b_8 - 17$	1082.8	1104.9	—	1112.5
b_8	1099.6	1121.8	1145.4	1129.6
$[M + H - 17]$	1100.7			
Parent	1117.5	1139.5	1163.5	1147.6

[a] —indicates not observed.
[b] All fragments from the $[M + Na]^+$ species retain the Na cation replacing a proton; $[M + H - 35]^+$ and $[M + H - 45]^+$ must also involve rearrangement from the $[M + H - 17]^+$ to enable subsequent loss of either H_2O or CO, respectively.

the fragmentation pattern corresponds to the MS/MS of a C-terminally amidated derivative of species **2** (isobaric with compound **1**). We could exclude possibility (ii) on the basis that no evidence for dcΔDOPA bound to an N-terminal Phe was observed in the ^1H NMR spectrum, nor was such an impurity detected by two orthogonal methods of analysis (analytical RP-HPLC and AU-PAGE).

Based on the previous work of Vachet et al. who observed a shift of the C-terminal residue to the N-terminus in the leucine enkephalin a_4 fragment ion that occurred together with elimination of ammonia [2], we propose that the C-terminal dcΔDOPA residue in plicatamide is transferred to the N-terminus. Previously, it was proposed that this reaction can occur either concerted with or stepwise after loss of NH_3 from the protonated molecule ion [2]. We carried out deuterium exchange experiments and MS/MS measurements to confirm the assignment of the z_8 fragment ion to be due to loss of ammonia (ND_3). In addition, MS^3 analysis of the z_8 precursor shows an intense series of fragment ions corresponding with the β series extending from β_3 to β_6 (see Figure 2A and Table 2). The observation of this series of fragment ions suggests that the rearrangement occurs in a concerted fashion (i.e., the *m/z* 1100 fragment ion has undergone almost complete rearrangement from the structure of z_8 to that in which the dcΔDOPA residue is covalently attached to the N-terminus of the peptide). MS^3 analysis was also carried out on the *m/z* 963.6 (β_6) fragment ion generated from the plicatamide $[M + H]^+$. A number of intense N- and C-terminal fragment ions (see Figure 2B and Table 2) were observed. All fragment ions observed from the β_6 precursor were consistent with expected fragments formed from the rearranged structure, i.e., N-terminal fragment ions contain the dcΔDOPA, whereas C-terminal fragment ions do not contain the dcΔDOPA group (N.B., in the case of N-terminal fragment ions, we use α to indicate cleavage at the equivalent bond to an a type cleavage in recognition of the fact that the rearrangement has added the dcΔDOPA group to the N-terminal fragment, just as we used β to indicate b type cleavages, otherwise the nomenclature confirms to that previously proposed [16]. Because the C-terminal dcΔDOPA residue of plicatamide is already decarboxylated, we envis-



Scheme 1. Proposed gas phase reaction scheme **(A)** concerted and **(B)** stepwise to generate N-terminally rearranged fragment ion [2] isobaric with the z_8 fragment ion.

age that the protonated plicatamide $[M + H]^+$ closely resembles an immonium ion enabling the rearrangement analogous to that observed by Vachet et al. [2]. We note that [Tyr^8] plicatamide and [DOPA^8] plicatamide do not undergo extensive loss of NH_3 and that the rearrangement to form the β series is not observed (see Table 1). It would appear that after ionization, the decarboxylated C-terminal protonated peptide ion is an exceedingly reactive intermediate for rearrangement. The comparatively long residence times of ions in the ion trap instrument has been proposed to increase the reaction time scale for such rearrangements [2]. We observed that the β series of fragment ions formed from

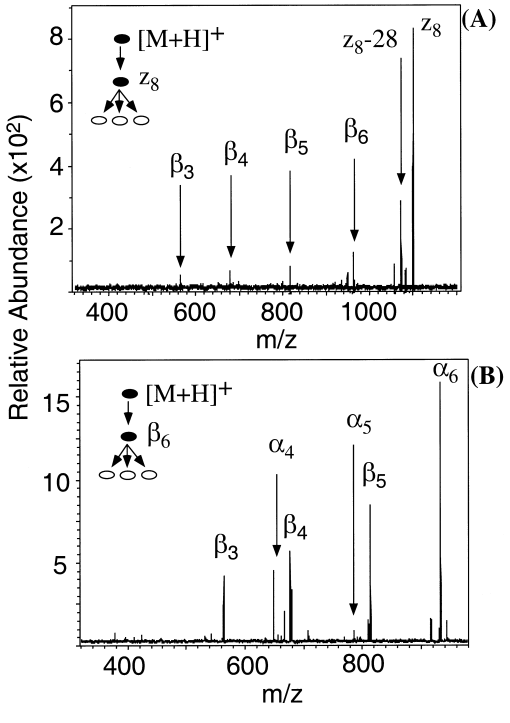


Figure 2. Compares the electrospray ion trap MS^3 analysis of the **(A)** z_8 and **(B)**, β_6 **fragment ions generated from the plicatamide** $[M + H]^+$.

Table 2. Assignment of fragment ions observed from the MS^3 analysis of the z_8 and β_6 fragment ions generated from the plicatamide $[M + H]^+$

Assignment	z_8	β_6
β_3	566.4	566.3
α_4	—[a]	651.4
β_4	679.4	679.6
$z_5 + 2$	—	669.3
$y^5 + 1$	—	682.6
$x^5 + 1$	—	710.4
α_5	—	789.0
z_6	—	812.6
β_5	816.6	816.6
$\alpha_6 - 17$	—	919.3
α_6	935.4	935.4
$\beta_6 - 17$	—	946.1
Not assigned	949.5	—
β_6	963.6	—
$a_7 - 17$	1056.5	—
a_7	1072.5	—
$b_7 - 17$	1083.8	—
Parent	1100.7	963.6

[a] —indicates not observed.

the z_8 precursor increases in intensity as the residence time of the ions in the ion trap is extended from 40 to 200 ms. The absence of the β-series of fragment ions from the $[M + Na]^+$ ion may indicate that transfer of a proton is critical for the rearrangement or that the cationized precursor ion does not have sufficient energy to surmount the activation energy barrier for the rearrangement reaction.

Oxidatively decarboxylated peptides two to four residues in length have been found in a variety of marine organisms including ascidians [17–19] and sponges [20–23]. However, to our knowledge, plicatamide is the first of this class of oxidatively decarboxylated peptides in which this rearrangement has been observed. This observation may prove important for future analysis of modified peptides and larger natural products, in particular those containing oxidatively decarboxylated C-terminal residues.

Conclusion

When interpreting MS/MS spectra, rearrangement ions can limit the ability to deduce the sequence of an unknown peptide. In particular, when carrying out the analysis with an ion trap instrument, the possibility of rearrangements which result in fragment ions not consistent with the proposed precursor structure must be recognized. Here we document encountering a rearrangement when characterizing plicatamide, a previously uncharacterized peptide with a decarboxylated DOPA residue at the C-terminus, using MS/MS. Fortuitously, in this case, the rearrangement reaction had been characterized [2] and the rearranged fragment ions were appropriately assigned.

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